General Overview of
Secondary Metabolism

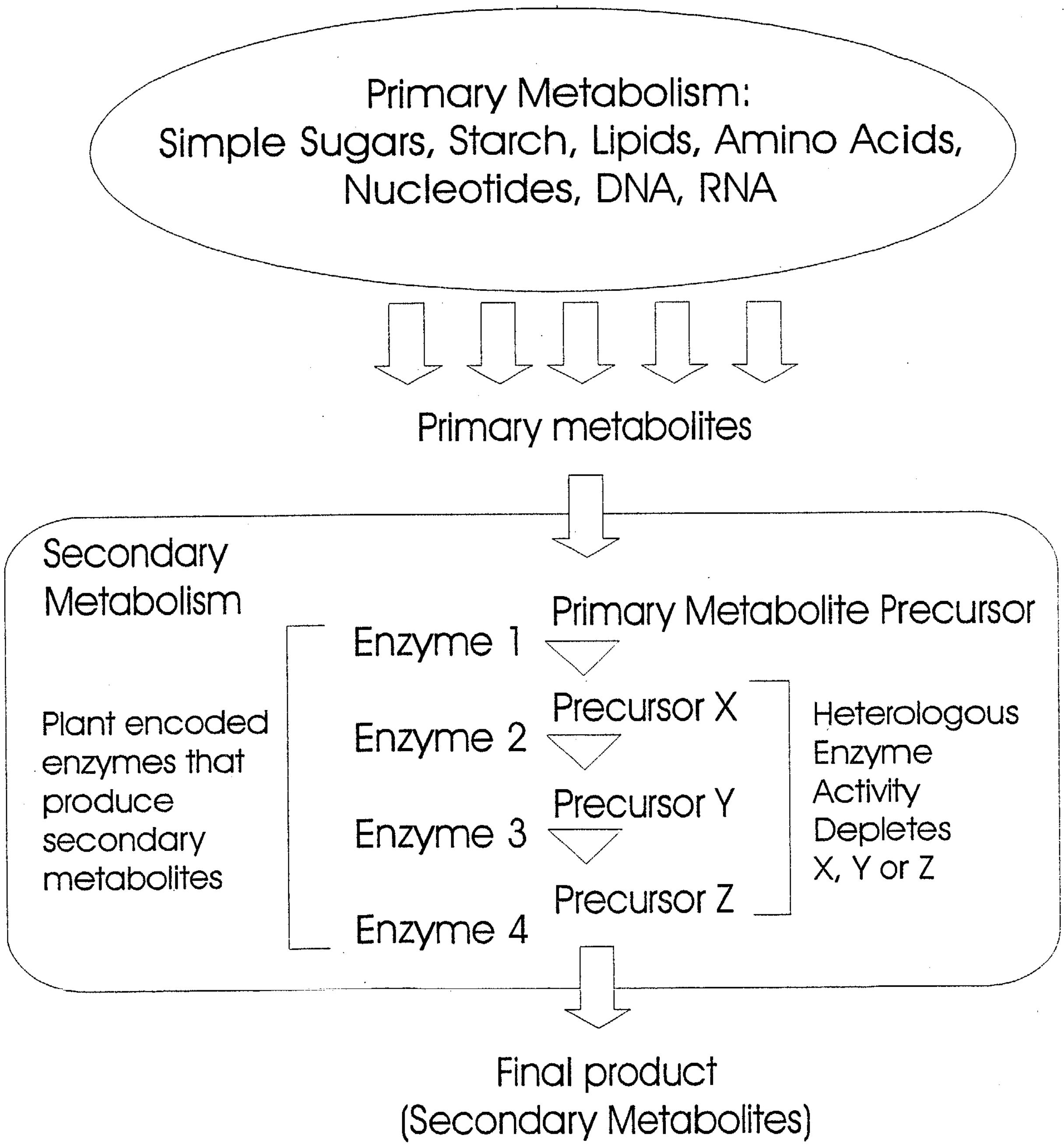


FIG. 1

General Phenylpropanoid Metabolism

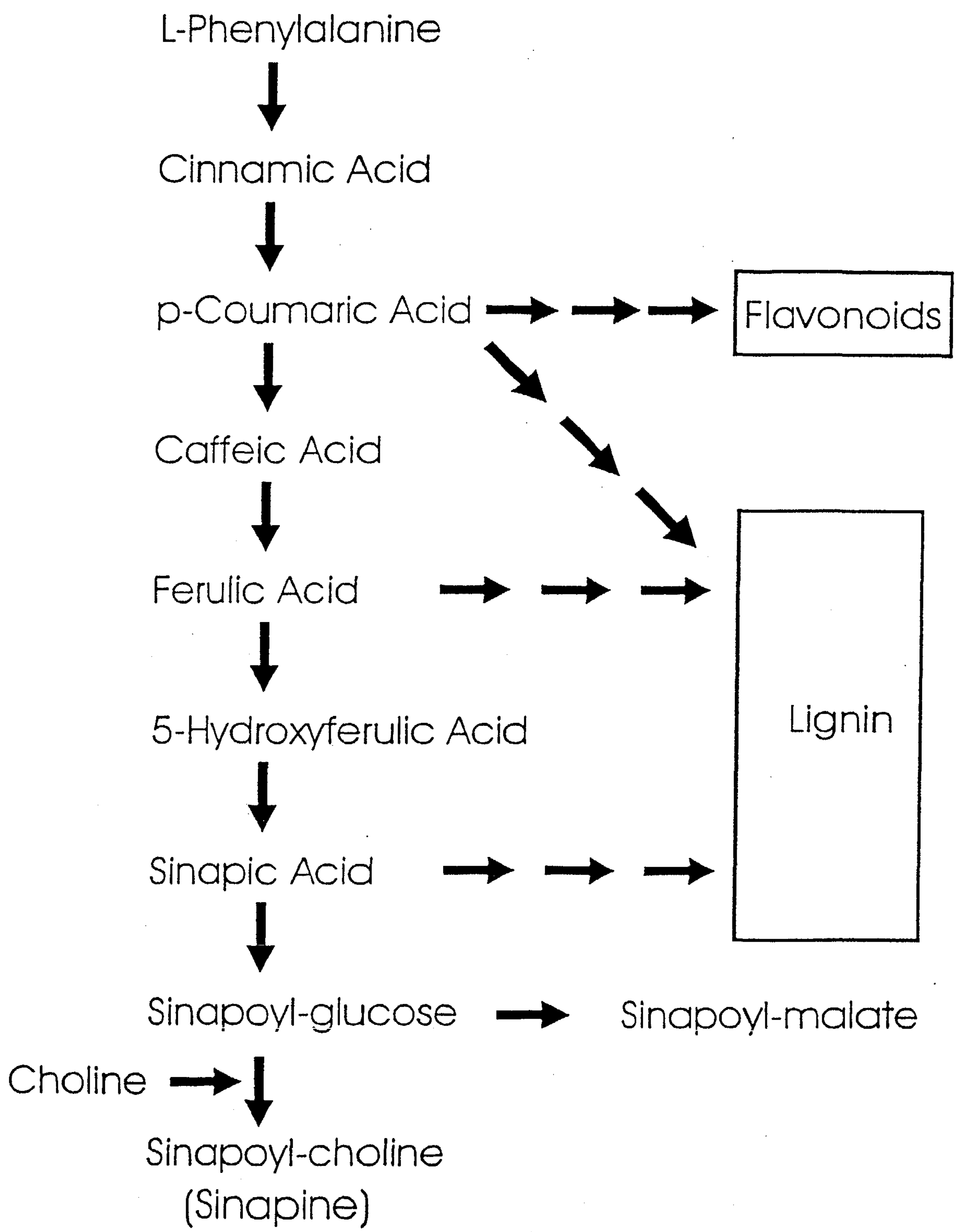
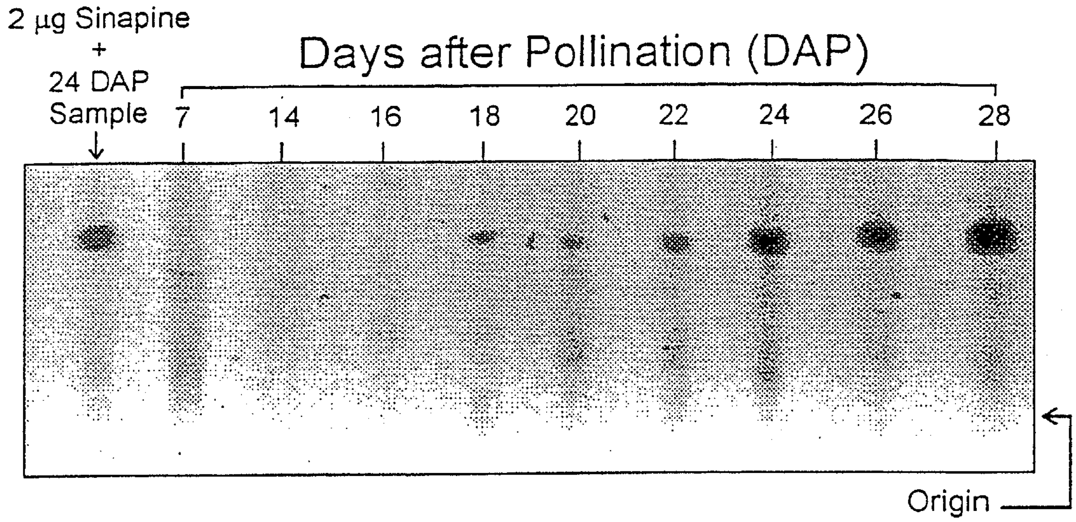


FIG. 2

Sinapine in *Brassica napus* Seeds



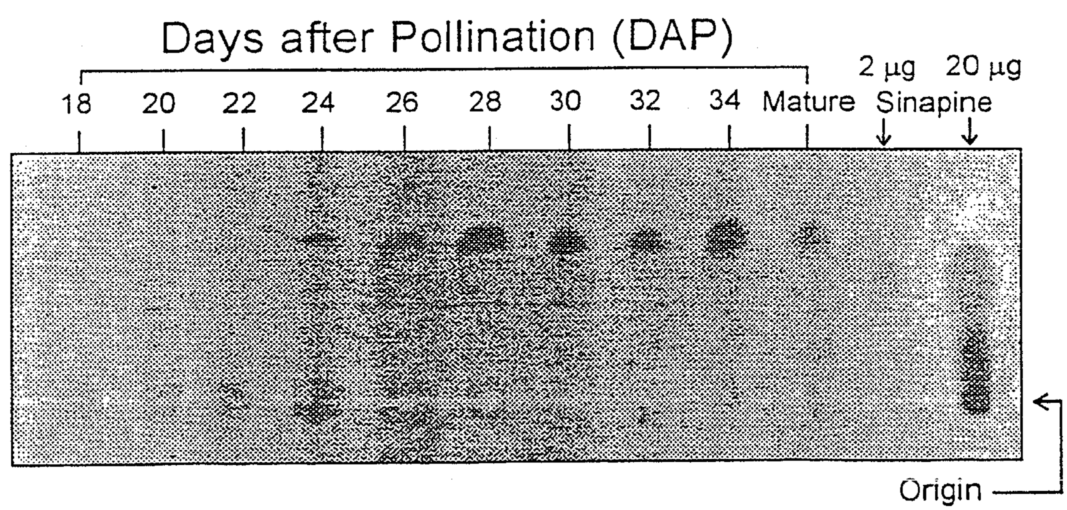


FIG. 3

Sinapine Accumulation in Developing Seeds of *B. napus* cv Westar

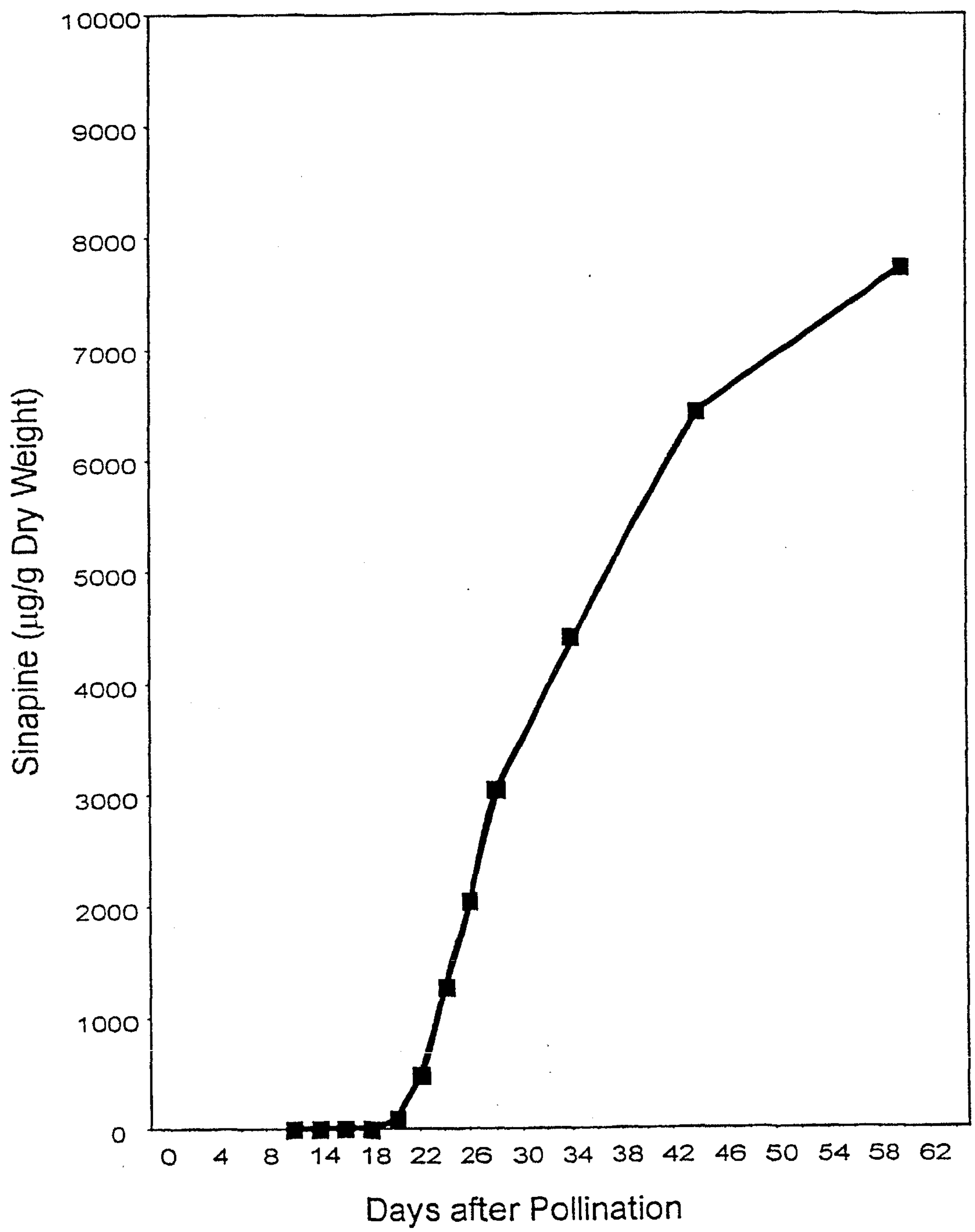


FIG. 4

Sinapine Synthesis in *Brassica napus* Seeds incubated with 14-C Choline

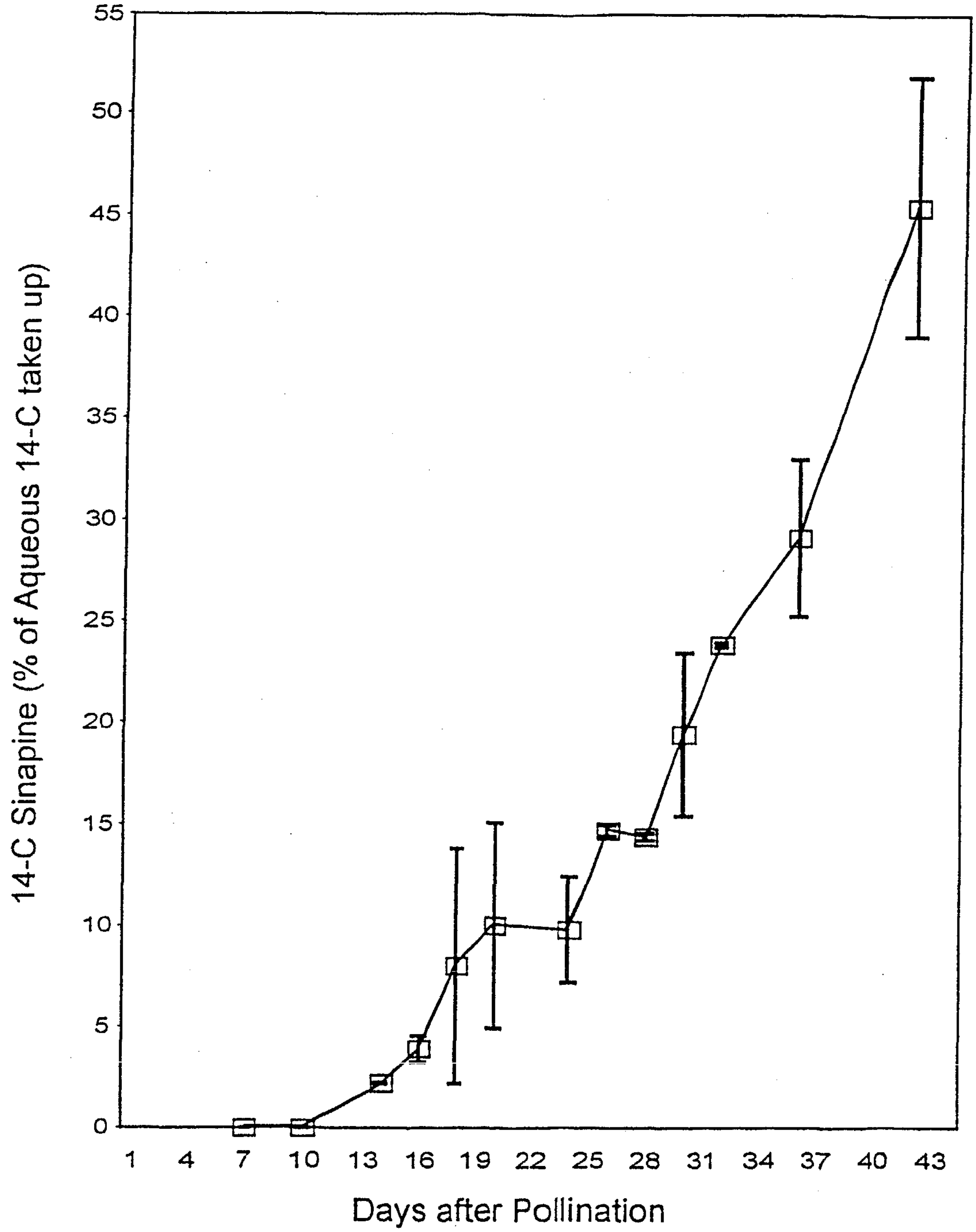


FIG. 5

Sinapine Synthesis in *Brassica napus* Seeds infiltrated with 14-C Choline

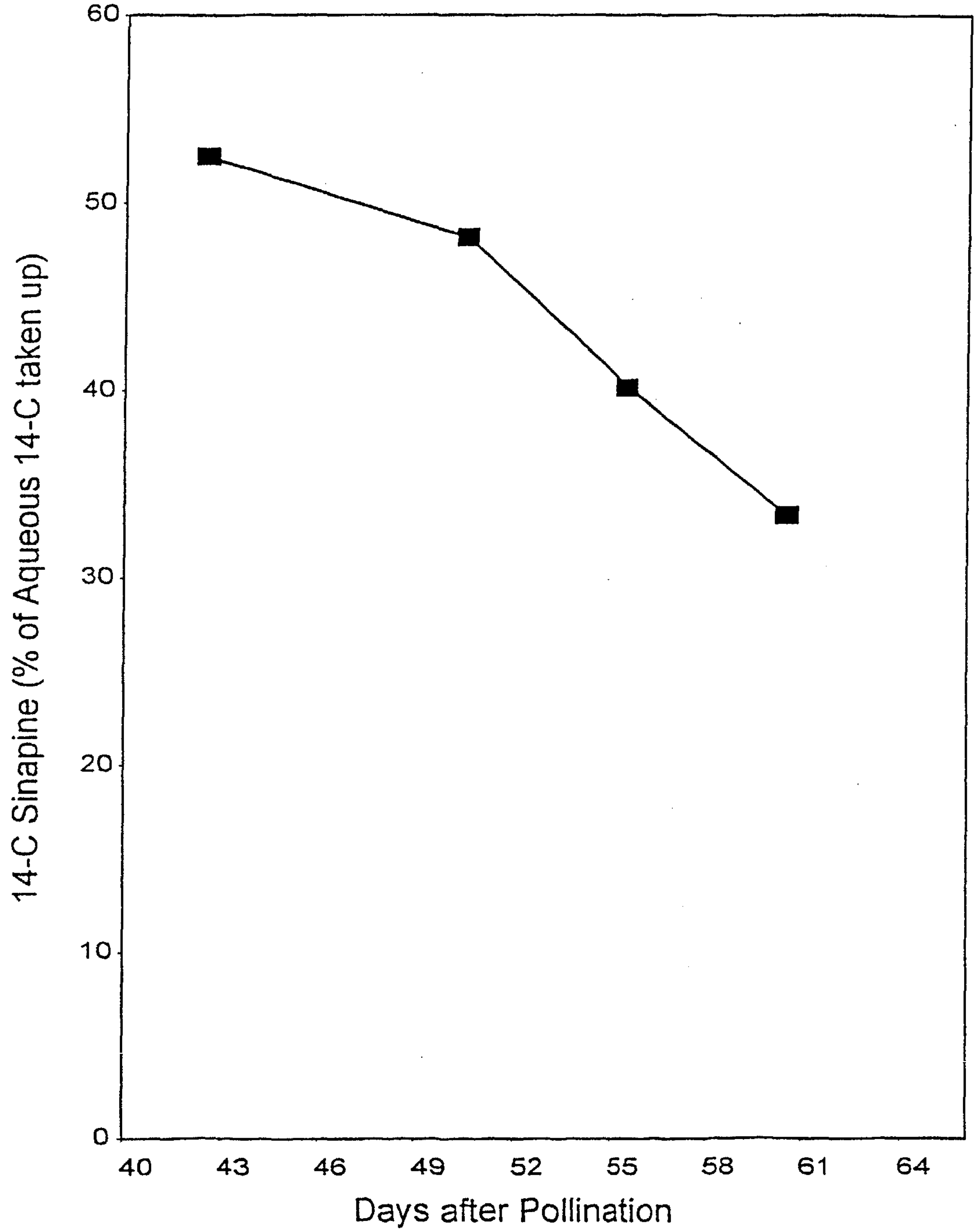


FIG. 6

Location of Radiolabelled Sinapine in *B. napus* seed

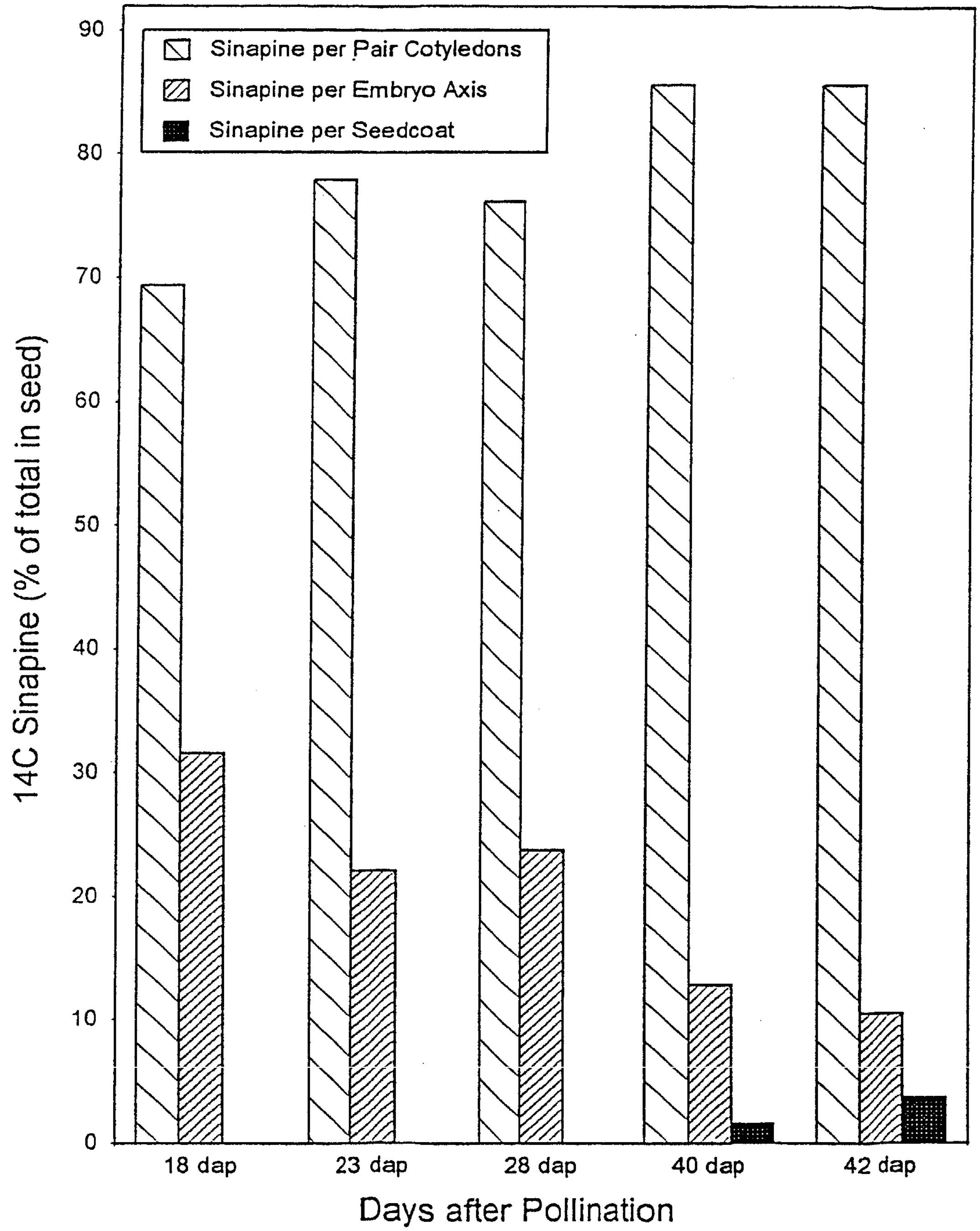


FIG.7

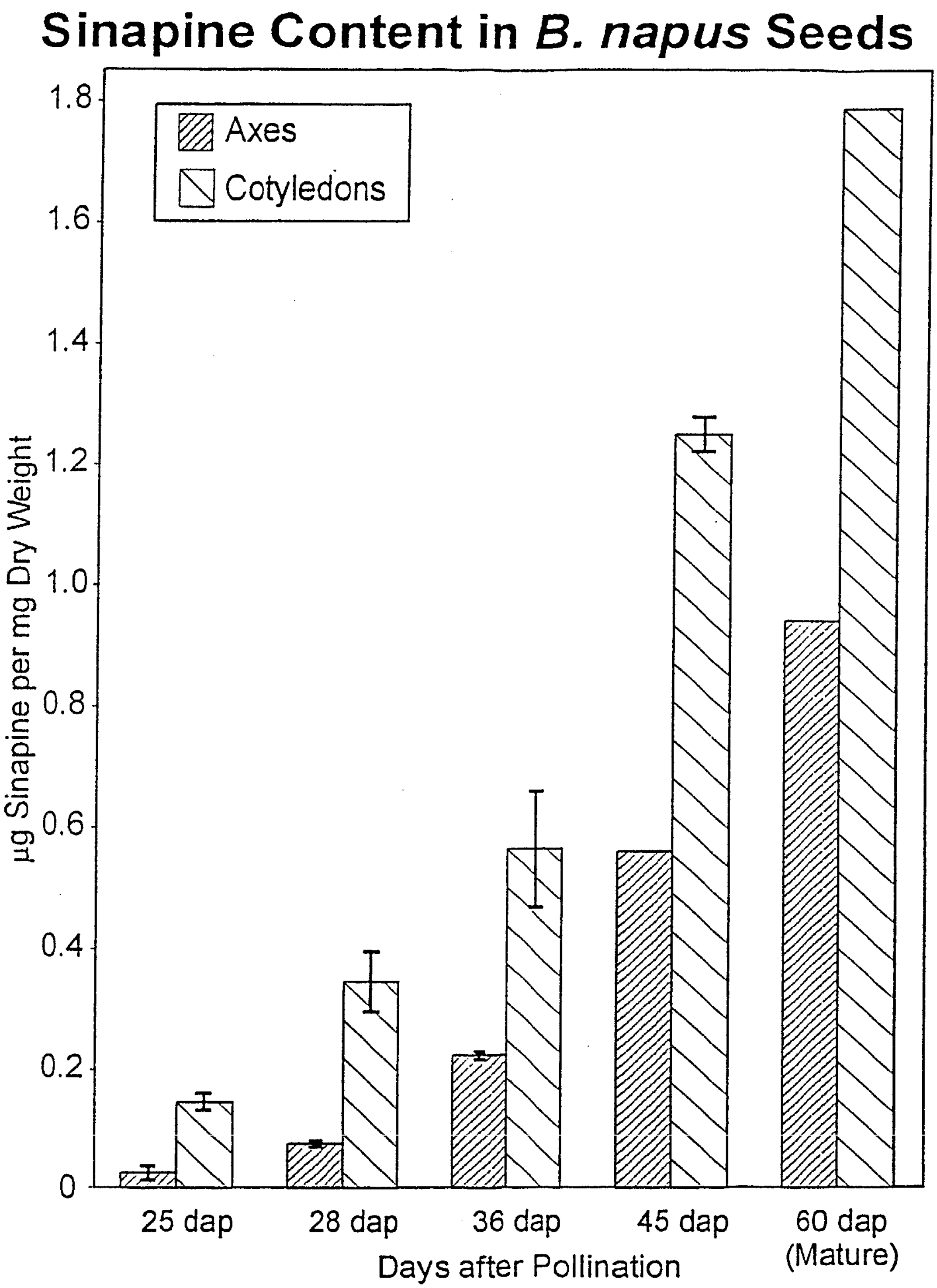

Sinapine Content in *B. napus* Seeds
Axes
Cotyledons
µg Sinapine per mg Dry Weight
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
25 dap
28 dap
36 dap
45 dap
60 dap (Mature)
Days after Pollination

FIG. 8

Sinapine Content in *B. napus* Seeds

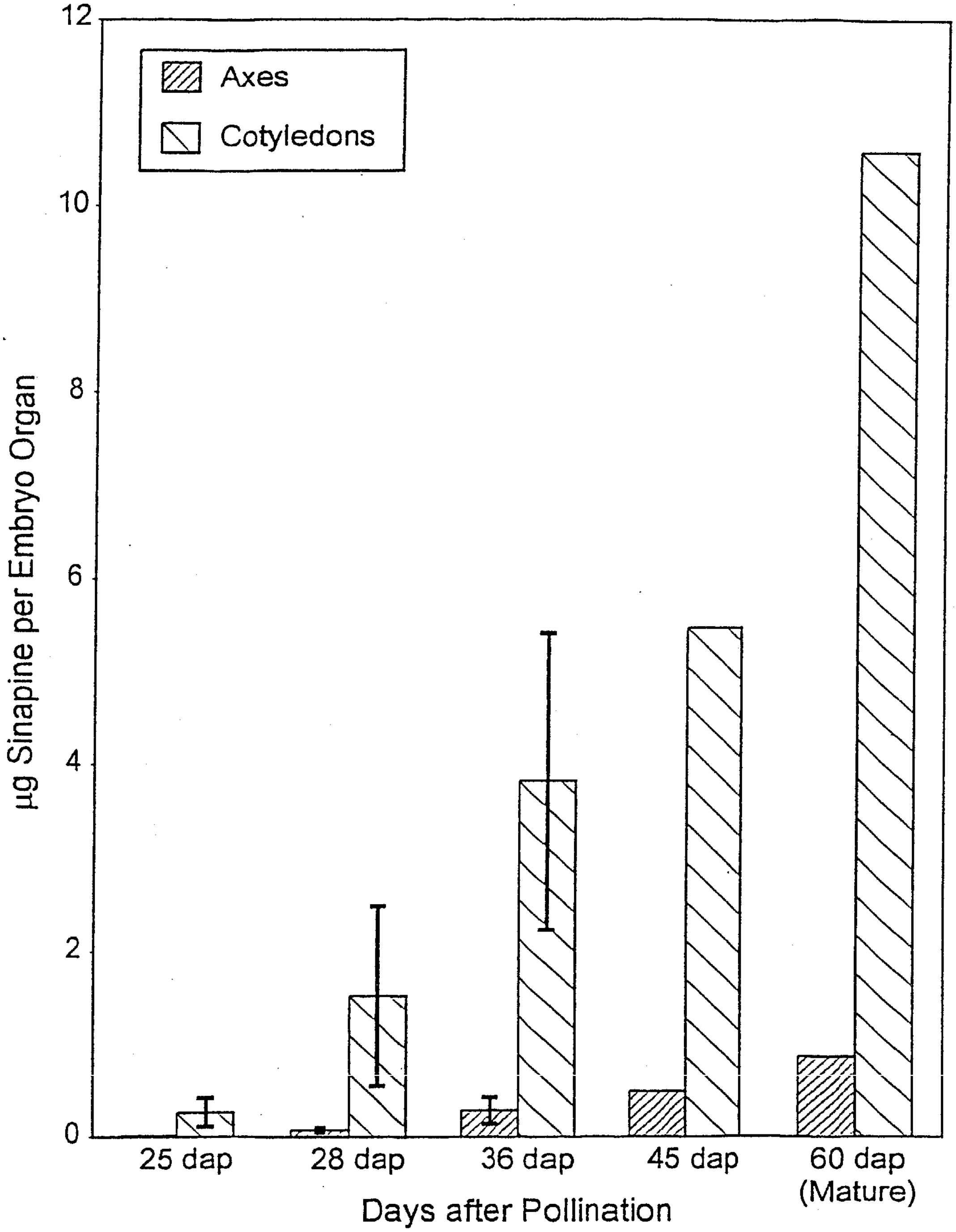


FIG. 9

1	ATGCACATCG	ACAACGTCGA	AAACCTCAAC	GACCGCGAGT	TCGACTACAT
51	CATCATCGGC	GGCGGTTCCG	CCGGAGCGGC	AGTCGCCGCC	CGCCTGAGCG
101	AGGAGCCCAC	CGTGTCCGTG	GCGCTGGTGG	AGGCCGGCCC	GGACGACCGC
151	GGCGTTCCCG	AGGTACTGCA	GCTCGACCGC	TGGATGGAGC	TGCTGGAATC
201	CGGCTACGAC	TGGGACTACC	CGATCGAACC	GCAGGAGAAC	GGCAACTCCT
251	TCATGCGCCA	CGCCCGCGCG	AAGATCATGG	GTGGCTGCTC	CAGCCACAAC
301	TCCTGCATCG	CCTTCTGGGC	CCCGCGCGAA	GACCTGGACG	AGTGGGAGTC
351	CAAGTACGGC	GCCACCGGCT	GGAACGCTGA	GTCCGCCTGG	CCGCTGTACC
401	AGCGGCTGGA	GACCAACGAG	GACGCCGGCC	CGGACGCGCC	GCACCACGGC
451	GACTCAGGCC	CGGTGCACCT	GATGAACGTG	CCCCCGGCGG	ACCCCGCCGG
501	CGTCGCACTC	CTGGACGCCT	GCGAACAGGC	AGGCATTCCG	CGCGCGAAGT
551	TCAACACCGG	CACCACCGTG	ATCAATGGCG	CCAACTTTTT	CCAGATCACA
601	CGCCGCGCGG	ACGGCACCCG	TTCCTCCAGC	TCGGTCTCCT	ACATCCACCC
651	GATCATCGAG	CGCGGGAACT	TCACCCTGCT	GACCGGGTTG	CGCGCCCGGC
701	AACTGGTGTT	CGACGCGGAC	AAGCGCTGCA	CCGGCGTCGA	CGTTGTGGAC
751	TCGGCGTTCG	GCCGGACTCA	CCGGCTCTCC	GCGCGTTGCG	AGGTCATCCT
801	GTCCACCGGC	GCCATTGACT	CGCCTAAGCT	GCTCATGCTC	TCCGGCATCG
851	GCCCCGCCGC	GCACCTCGCC	GAGCACGGCG	TCGAGGTCCT	GGTCGACTCC
901	CCCGGTGTCG	GCGAGCACCT	GCAGGACCAC	CCCGAAGGCG	TCGTCCAGTT
951	CGAGGCCAAG	CAGCAGATGG	TGCAGACTTC	GACGCAGTGG	TGGGAGATCG
1001	GCATCTTCAC	CCCCACCGAG	AACGGCCTGG	ACCGCCCGGA	CCTGATGATG
1051	CACTACGGCT	CCGTCCCGTT	CGACATGAAC	ACCCTGCGGT	ACGGCTACCC
1101	CACCACGGAG	AACGGCTTCA	GCCTCACGCC	GAACGTCACG	CACGCCCGCT
1151	CCCGCGGCAC	CGTCCGGCTG	CGCAGCCGCG	ACTTCCGCGA	CAAGCCCGCC
1201	GTCGACCCGC	GGTACTTCAC	TGATCCGGAG	GGCCACGACA	TGCGCGTCAT
1251	GGTGGCCGGC	ATCCGCAAGG	CCCGTGAAAT	CGCCGCCCAG	CCTGCCATGG
1301	CCGAATGGAC	CGGCCGCGAG	CTCTCGCCCG	GCACCGAGGC	GCAGACCGAC

FIG. 10A

1351 GAGGAACTGC AGGACTACAT CCGCAAGACG CACAACACCG TTTACCACCC

1401 CGTCGGCACC GTCCGCATGG GACCAGCCGA CGACGACATG TCGCCGCTCG

1451 ACCCCGAGCT GCGGGTGAAG GGCGTGACCG GCCTGCGCGT CGCCGATGCC

1501 TCTGTCATGC CTGAACACGT CACGGTCAAT CCCAACATCA CCGTCATGAT

1551 GATCGGCGAA CGCTGCGCCG ACCTCATCCG CGCCAGCCGG ACCGGCGAAA

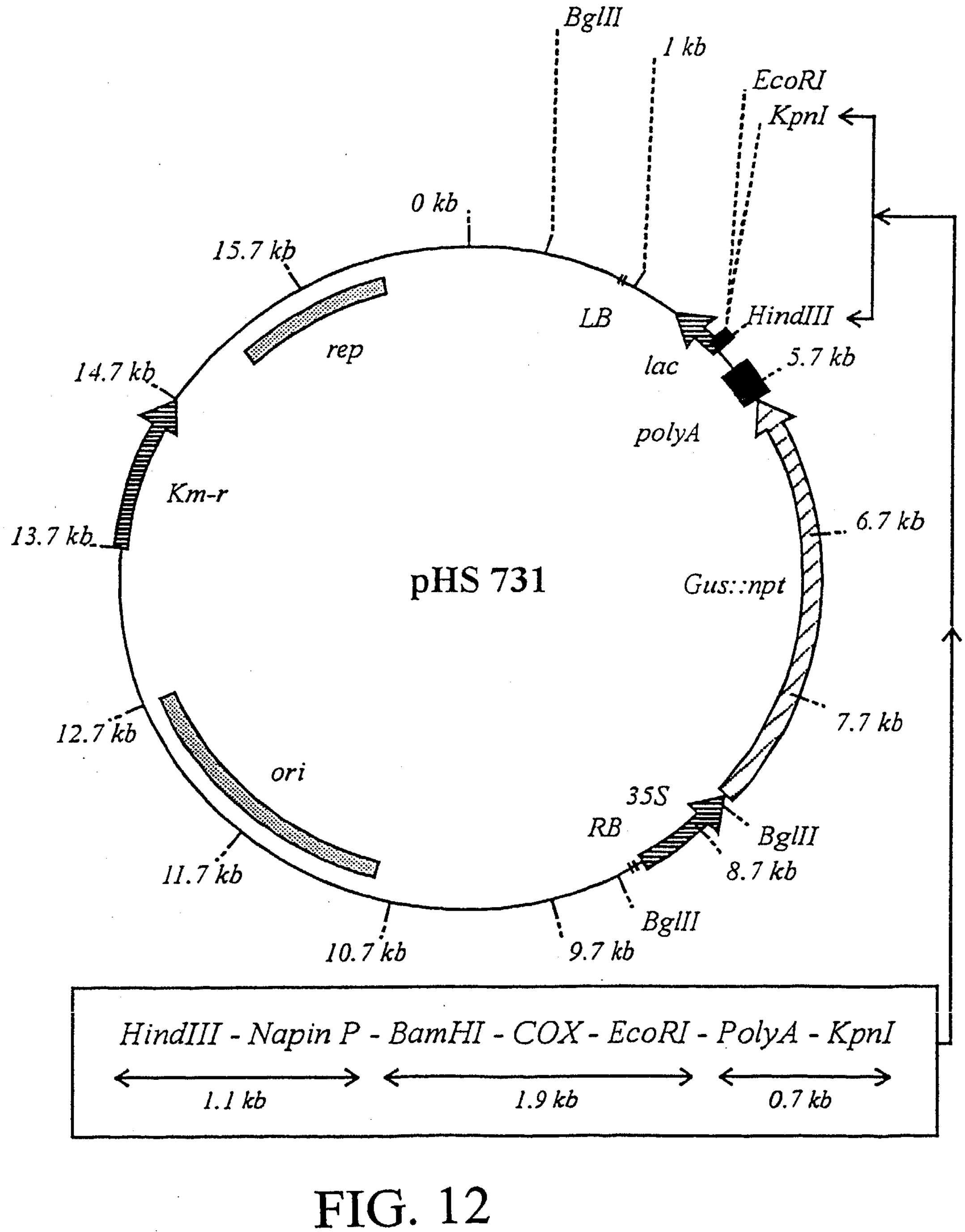
1601 CAACGACGGC GGAGGCGGAG CTCAGCGCGT CCCTCGCCTG A

FIG. 10B

Predicted amino acid sequence of choline oxidase open frame.

```
  1 MHIDNVENLN DREFDYIIIG GGSAGAAVAA RLSEEPTVSV ALVEAGPDDR
 51 GVPEVLQLDR WMELLESGYD WDYPIEPQEN GNSFMRHARA KIMGGCSSHN
101 SCIAFWAPRE DLDEWESKYG ATGWNAESAW PLYQRLETNE DAGPDAPHHG
151 DSGPVHLMNV PPADPAGVAL LDACEQAGIP RAKFNTGTTV INGANFFQIT
201 RRADGTRSSS SVSYIHPIIE RGNFTLLTGL RARQLVFDAD KRCTGVDVVD
251 SAFGRTHRLS ARCEVILSTG AIDSPKLLML SGIGPAAHLA EHGVEVLVDS
301 PGVGEHLQDH PEGVVQFEAK QQMVQTSTQW WEIGIFTPTE NGLDRPDLMM
351 HYGSVPFDMN TLRYGYPTTE NGFSLTPNVT HARSRGTVRL RSRDFRDKPA
401 VDPRYFTDPE GHDMRVMVAG IRKAREIAAQ PAMAEWTGRE LSPGTEAQTD
451 EELQDYIRKT HNTVYHPVGT VRMGPADDDM SPLDPELRVK GVTGLRVADA
501 SVMPEHVTVN PNITVMMIGE RCADLIRASR TGETTTAEAE LSASLA*
```

FIG. 11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EcoRI
KpnI
0 kb
15.7 kb
LB
HindIII
rep
lac
5.7 kb
14.7 kb
polyA
Km-r
6.7 kb
13.7 kb
pHS 731
Gus::npt
12.7 kb
7.7 kb
ori
35S
RB
BglII
8.7 kb
11.7 kb
BglII
10.7 kb
9.7 kb
HindIII - Napin P - BamHI - COX - EcoRI - PolyA - KpnI
1.1 kb
1.9 kb
0.7 kb

FIG. 12

Sinapine Content of Transgenic *Brassica napus* Seeds

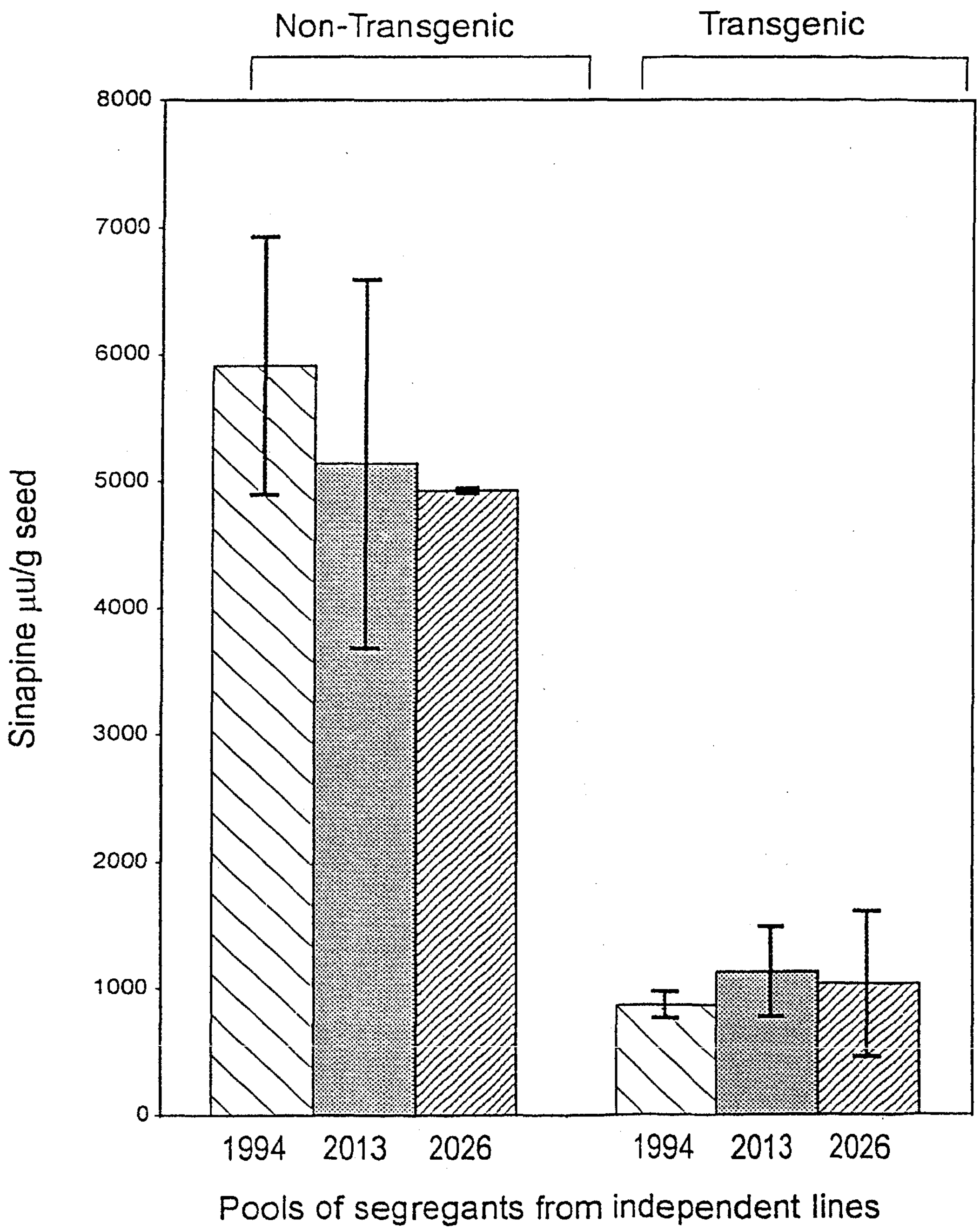
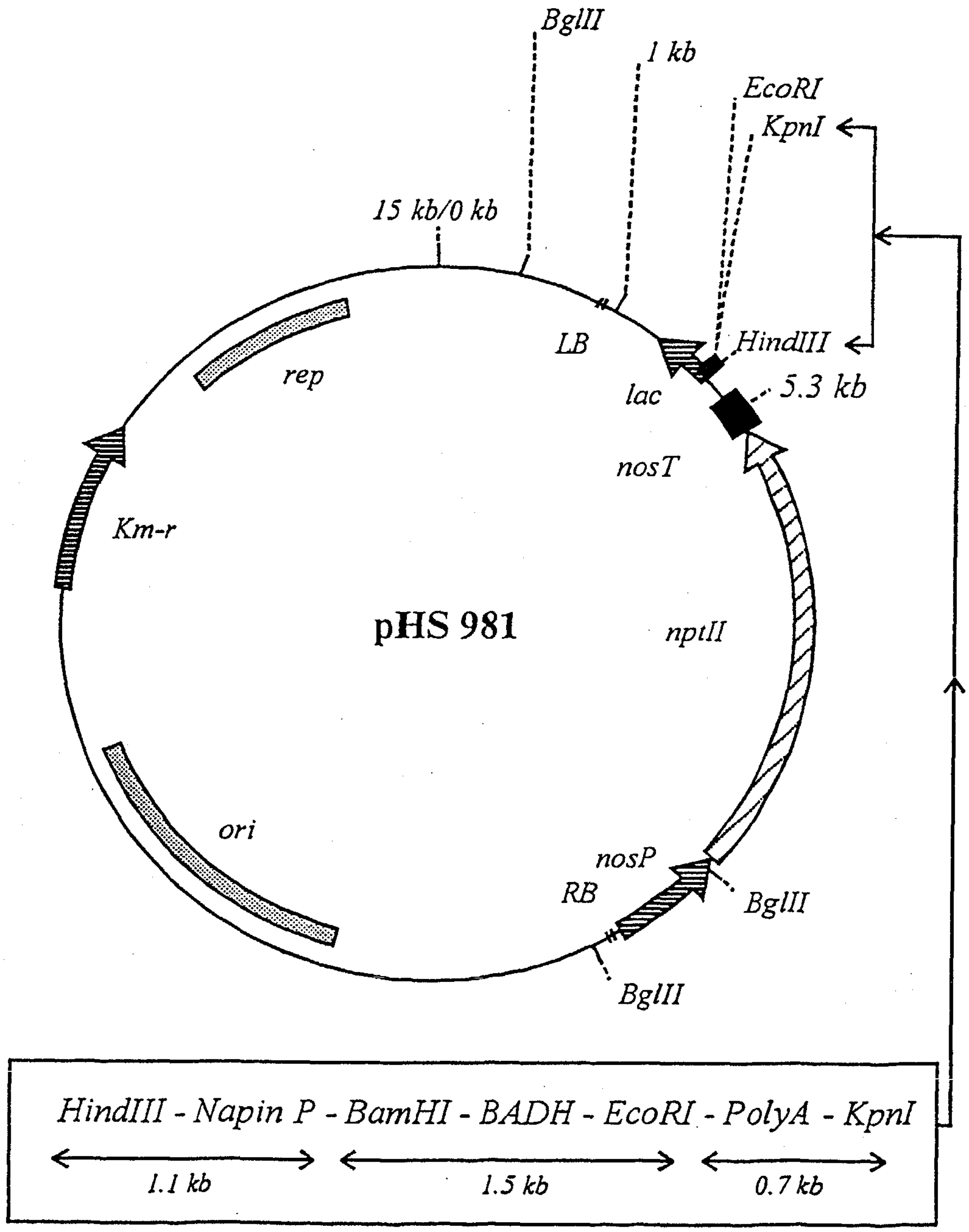


FIG. 13



BglII
1 kb
EcoRI
KpnI
15 kb/0 kb
LB
HindIII
rep
lac
5.3 kb
nosT
Km-r
pHS 981
nptII
ori
nosP
RB
BglII
BglII
HindIII - Napin P - BamHI - BADH - EcoRI - PolyA - KpnI
1.1 kb
1.5 kb
0.7 kb

FIG. 14

Reduction of Sinapine in Cox and Cox/BaDH lines

Plant line	Number of plants analyzed	Plant Genotype	Sinapine Absorbance Units	Sinapine Levels (Control = 100%)
2026 X 1534	15	Cox + Badh	13,878	13.6%
2013	23	Cox	19,874	19.5%
1994	26	Cox	23,344	22.9%
2026	27	control	101,856	100%

FIG. 15

Total Soluble Phenolic Content in Transgenic Lines

Plant line	Number of plants analyzed	Plant Genotype	Soluble Phenolic Absorbance Units	Soluble Phenolic Levels (Control = 100%)
2026 X 1534	15	Cox + Badh	103,828	69.0%
2013	23	Cox	98,222	78.0%
1994	26	Cox	97,370	77.3%
2026	27	control	125,882	100%

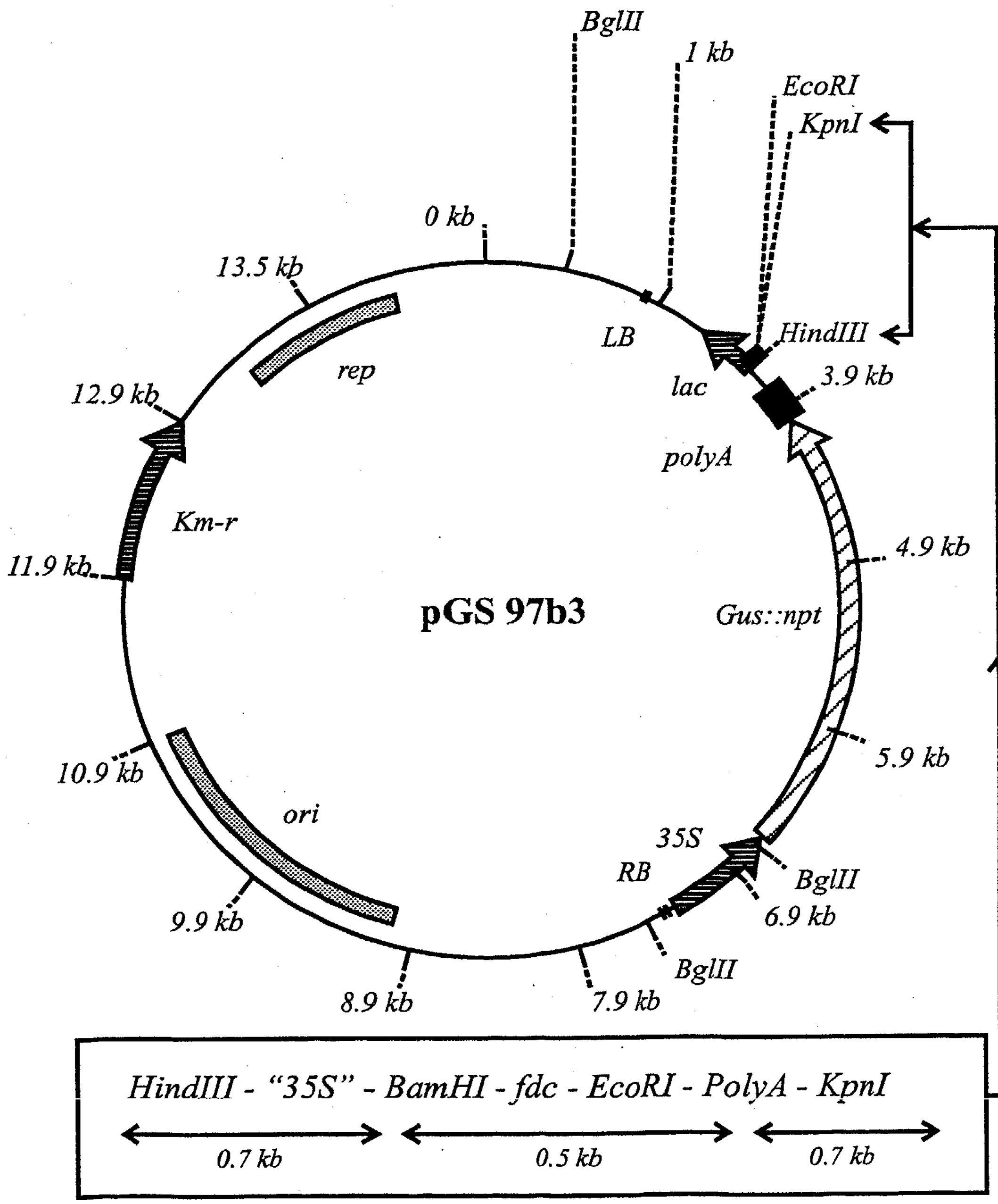
FIG. 16

1	ATGGACCAAT	TCGTGGGTCT	CCACATGATC	TACACATACG	AGAACGGTTG
51	GGAGTACGAA	ATCTACATCA	AGAACGACCA	CACAATCGAC	TACCGTATCC
101	ACAGTGGTAT	GGTGGGTGGT	AGGTGGGTGA	GGGACCAAGA	GGTGAACATC
151	GTGAAGCTCA	CAAAGGGTGT	GTACAAGGTG	AGCTGGACAG	AGCCAACAGG
201	TACAGACGTG	AGCCTCAACT	TCATGCCAGA	GGAGAAGAGG	ATGCACGGTG
251	TGATCTTCTT	CCCAAAGTGG	GTGCACGAGA	GGCCAGACAT	CACAGTGTGC
301	TACCAAAACG	ACTACATCGA	CCTCATGAAG	GAGAGCAGGG	AGAAGTACGA
351	GACATACCCA	AAGTACGTGG	TGCCAGAGTT	CGCTGACATC	ACATACATCC
401	ACCACGCTGG	AGTGAACGAC	GAGACAATCA	TCGCTGAgGC	TCCATACGAg
451	GGTATGACAG	ACGAgATCAG	GGCTGGTAgG	AAG	

FIG. 17

1 MDQFVGLHMI YTYENGWEYE IYIKNDHTID YRIHSGMVGG RWVRDQEVNI
51 VKLTKGVYKV SWTEPTGTDV SLNFMPEEKR MHGVIFFPKW VHERPDITVC
101 YQNDYIDLMK ESREKYETYP KYVVPEFADI TYIHHAGVND ETIIAEAPYE
151 GMTDEIRAGR K

FIG. 18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EcoRI
KpnI
0 kb
13.5 kb
LB
HindIII
rep
lac
3.9 kb
12.9 kb
polyA
Km-r
4.9 kb
11.9 kb
pGS 97b3
Gus::npt
5.9 kb
10.9 kb
ori
35S
RB
BglII
6.9 kb
9.9 kb
BglII
8.9 kb
7.9 kb
HindIII - "35S" - BamHI - fdc - EcoRI - PolyA - KpnI
0.7 kb
0.5 kb
0.7 kb

FIG. 19

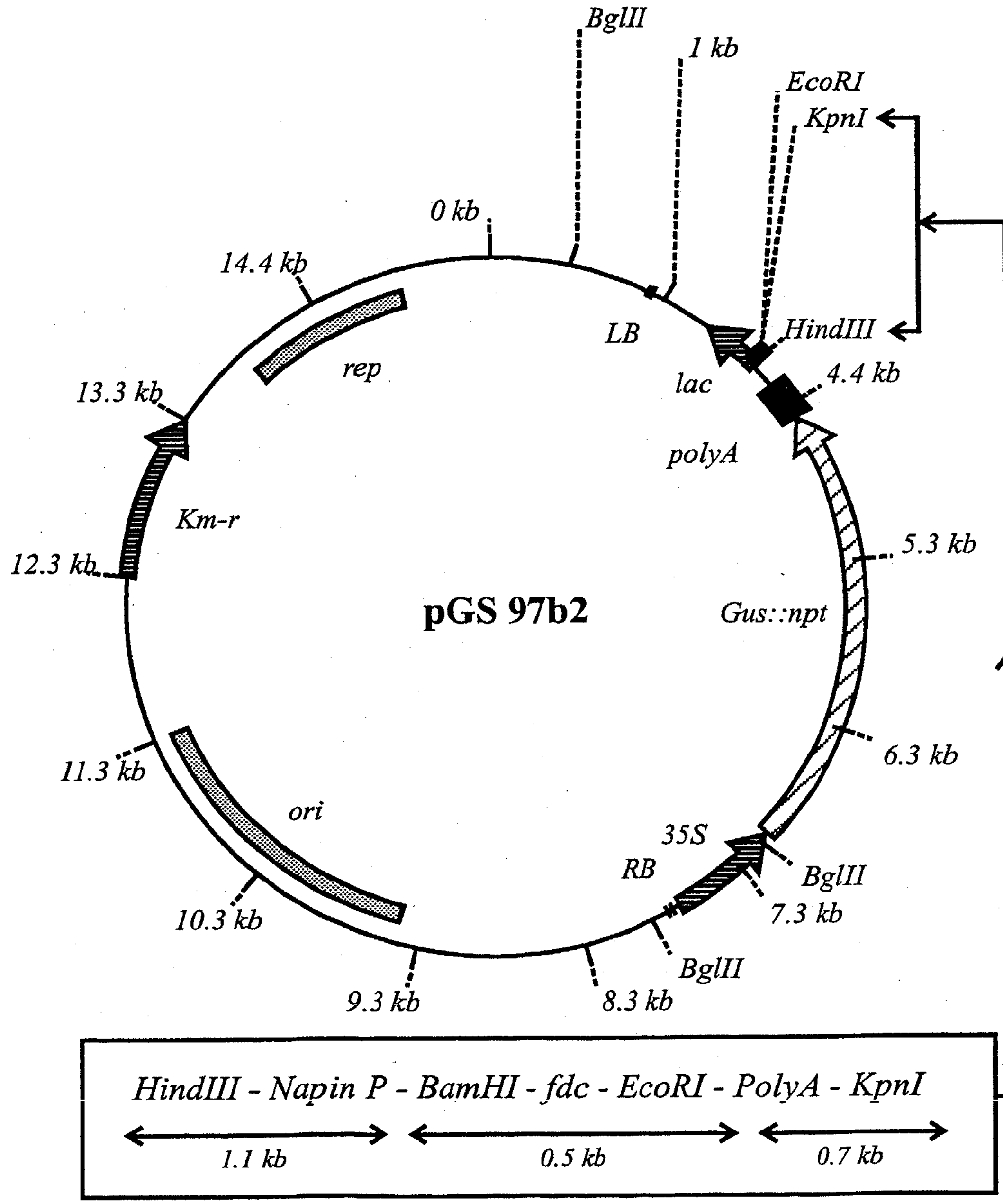

BglII
1 kb
EcoRI
KpnI
0 kb
14.4 kb
LB
HindIII
rep
lac
4.4 kb
13.3 kb
polyA
Km-r
5.3 kb
12.3 kb
pGS 97b2
Gus::npt
11.3 kb
6.3 kb
ori
35S
RB
BglII
7.3 kb
10.3 kb
BglII
9.3 kb
8.3 kb
HindIII - Napin P - BamHI - fdc - EcoRI - PolyA - KpnI
1.1 kb
0.5 kb
0.7 kb

FIG. 20

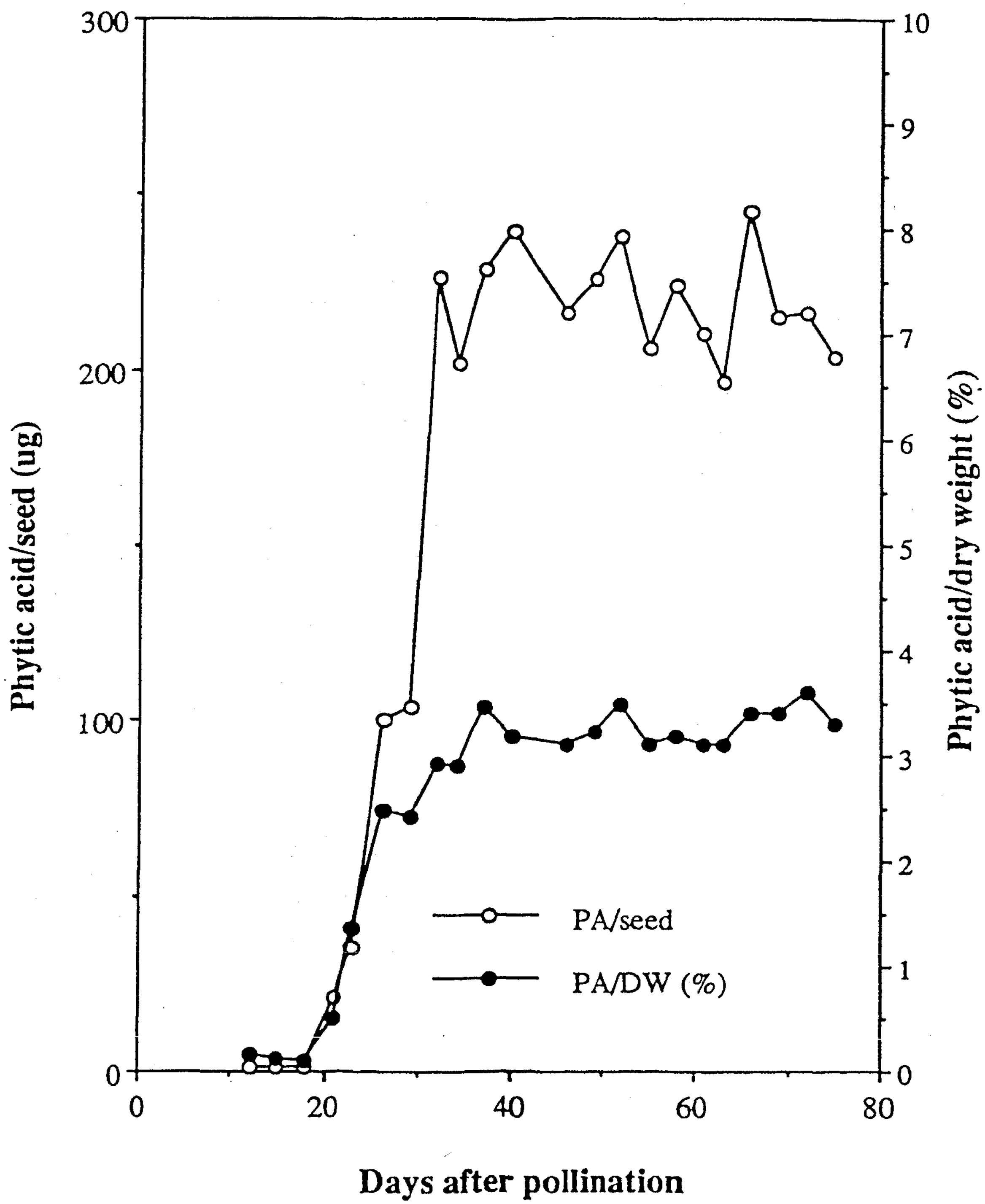

300
200
100
0
10
9
8
7
6
5
4
3
2
1
0
0
20
40
60
80
Phytic acid/seed (ug)
Phytic acid/dry weight (%)
Days after pollination
PA/seed
PA/DW (%)

FIG. 21

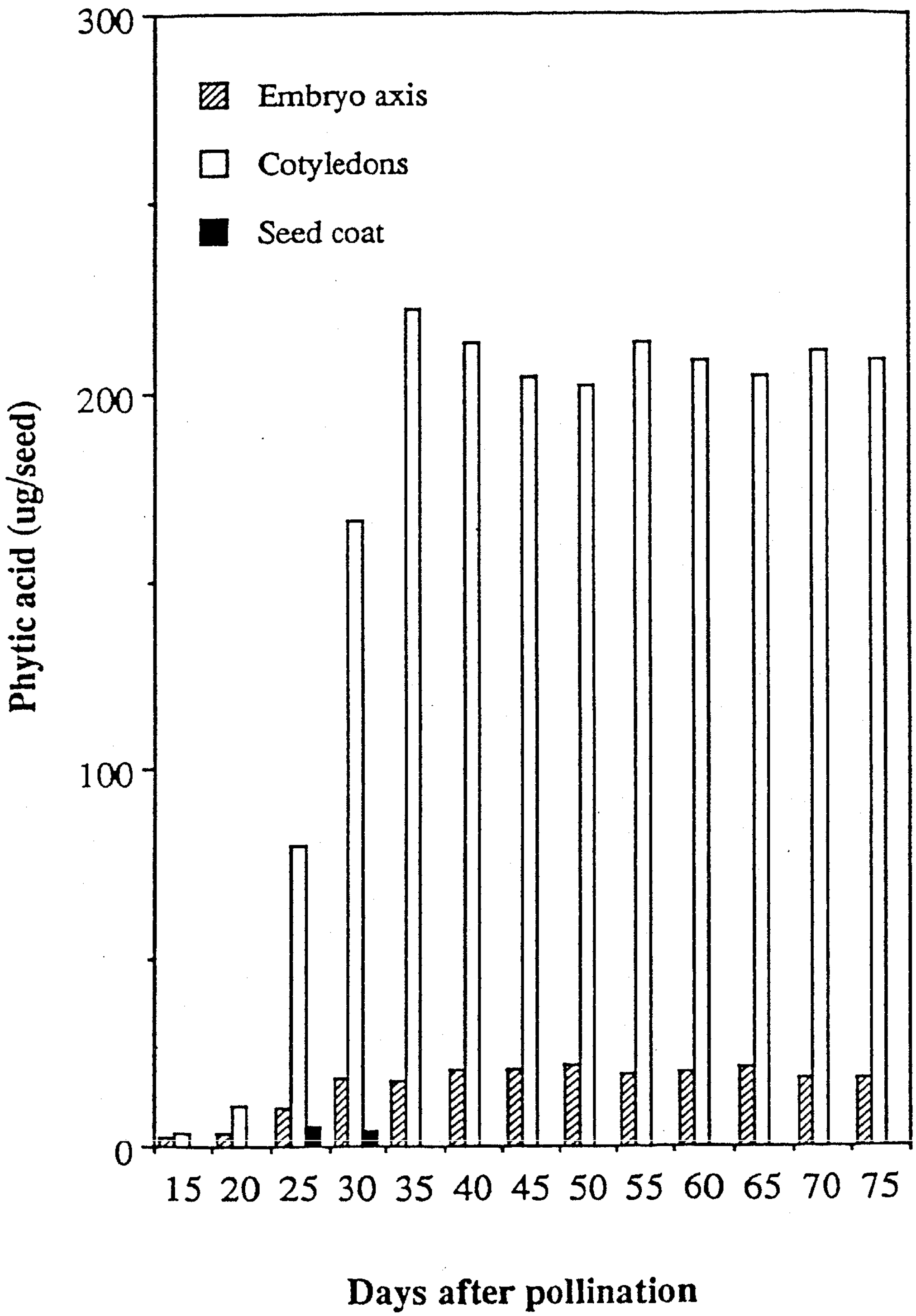

300
200
100
0
Embryo axis
Cotyledons
Seed coat
Phytic acid (ug/seed)
15
20
25
30
35
40
45
50
55
60
65
70
75
Days after pollination

FIG. 22

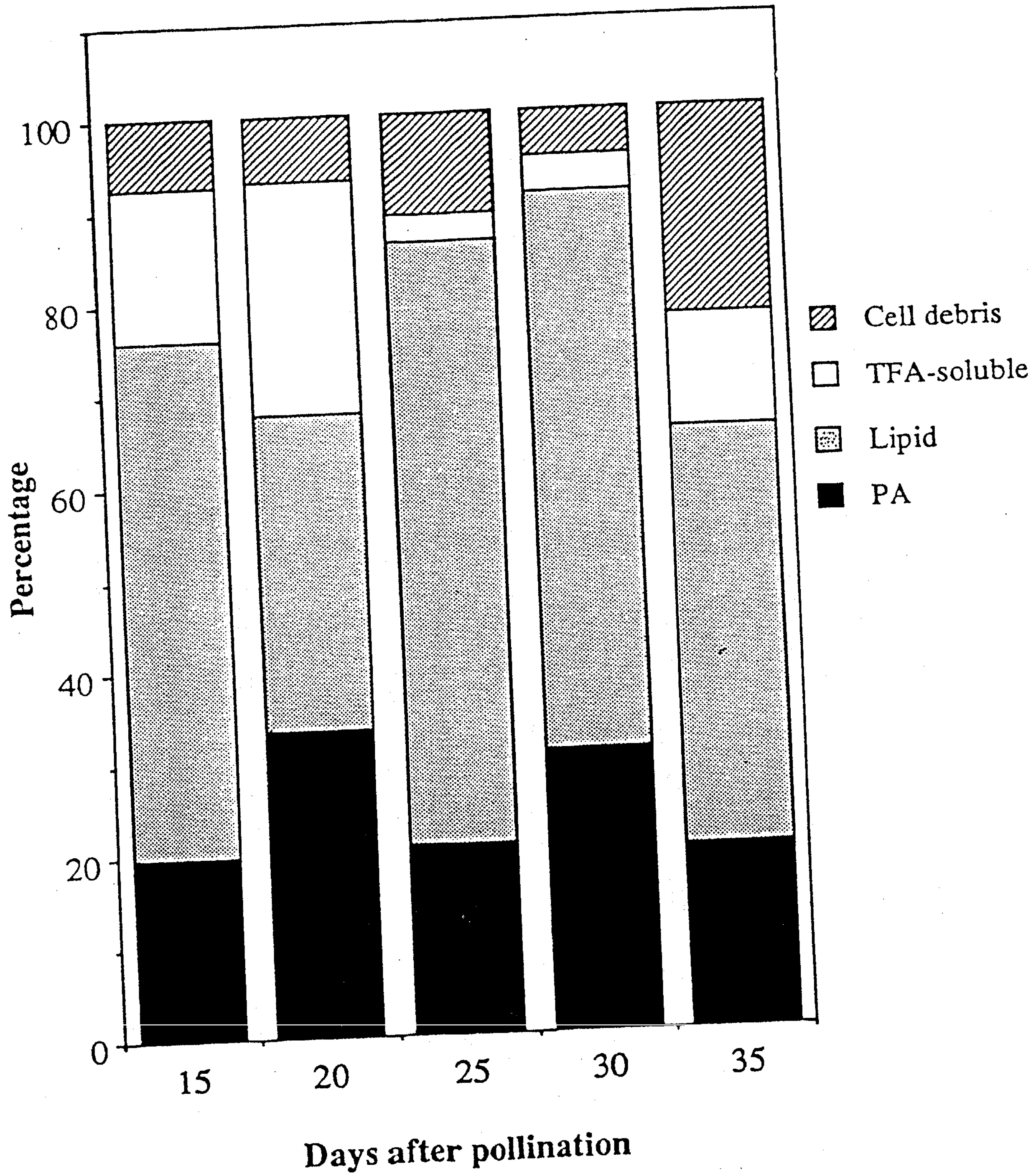

100
80
60
40
20
0
Percentage
15
20
25
30
35
Days after pollination
Cell debris
TFA-soluble
Lipid
PA

FIG. 23

```
cipimtla : AAAAAAAAAATTTTACTTCTCTGTTTTACCAAAAAGAGAAAAAAAAAATGACTACTTACAC
   imtsp : ..............................................--------------

cipimtla : CAATGGCAACTACACACAACCAAAAACCCTAGACAAAGATGAACAATTAGCTGGTTTGGC
   imtsp : ------------------------------------------------------------

cipimtla : AGTGACATTAGCAAATGCAGCTGCTTTTCCAATGATCCTGAAATCAGCCTTTGAGCTAAA
   imtsp : ------------------------------------------------------------

cipimtla : AATCCTTGACATATTCTCAAAAGCAGGGGAAGGCGTGTTTGTATCGACTTCTGAGATCGC
   imtsp : ------------------------------------------------------------

cipimtla : TAGCCAAATCGGGGCAAAGAACCCTAATGCCCCGGTGTTGTTGGACCGGATGCTCCGGCT
   imtsp : ------------------------------------------------------------

cipimtla : CCTGGCTAGCCACTCTGTGTTAACATGCAAGCTCCAAAAGGGTGAGGGTGGTTCTCAAAG
   imtsp : ------------------------------------------------------------

cipimtla : GGTGTATGGTCCAGCTCCCCTTTGCAACTATCTTGCTAGTAATGATGGTCAAGGCTCTCT
   imtsp : ------------------------------------------------------------

cipimtla : TGGCCCTTTGCTTGTTTTGCATCATGACAAGGTCATGATGGAGAGTTGGTTTCACTTGAA
   imtsp : ------------------------------------------------------------

cipimtla : TGATTACATACTAGAAGGAGGTGTTCCATTCAAGCGCGCTCATGGGATGATCCAATTCGA
   imtsp : ------------------------------------------------------------

cipimtla : CTACACTGGGACTGATGAAAGGTTCAATCATGTGTTCAACCAAGGGATGGCACACCACAC
   imtsp : ------------------------------------------------------------

cipimtla : TATCCTGGTCATGAAGAAGCTCCTTGACAACTACAATGGGTTTAATGATGTCAAGGTCCT
   imtsp : ------------------------------------------------------------

cipimtla : AGTTGATGTGGGTGGTAACATTGGTGTCAATGTGAGCATGATCGTCGCTAAGCATACTCA
   imtsp : ------------------------------------------------------------

cipimtla : CATTAAGGGCATCAACTATGACTTGCCTCATGTCATTGCTGATGCTCCTTCTTACCCCGG
   imtsp : ------------------------------------------------------------

cipimtla : TGTGGAGCATGTTGGTGGTAACATGTTTGAGAGCATACCACAAGCAGATGCCATTTTCAT
   imtsp : ------------------------------------------------------------

cipimtla : GAAGTGGGTGTTGCATGATTGGAGCGACGAGCATTGCGTGAAGATACTCAACAAGTGCTA
   imtsp : ------------------------------------------------------------

cipimtla : TGAGAGCCTGGCAAAGGAGGGAAGATCATCCTTGTGGAATCGCTTATACCAGTAATCCC
   imtsp : ------------------------------------------------------------

cipimtla : AGAAGACAACCTCGAATCACACATGGTGTTTAGCCTTGATTGCCACACTTTGGTGCACAA
   imtsp : ------------------------------------------------------------
```

FIG. 24

```
cipimtla : CCAAGGTGGAAAAGAGAGATCAAAGGAGGATTTTGAAGCCTTAGCTTCCAAGACTGGCTT
   imtsp : ------------------------------------------------------------

cipimtla : CTCTACAGTTGATGTCATTTGCTGTGCCTATGACACTTGGGTCATGGAGCTCTACAAGAA
   imtsp : ------------------------------------------------------------

cipimtla : GTGATTCAAGCTCTAAATGCTGTGTTGTTGTCATTGTTGCTAGCCCAAGTAGCTAGCTAG
   imtsp : ------------------------------------------------------------

cipimtla : CTGGTTAAAATTTCTCCTACCTAGCATTTGTTTTATGGCTAAGTTGAGGAGATTCATGTA
   imtsp : ------------------------------------------------------------

cipimtla : TTGTAAATGTTGTGTTTGGGTTTGGGTTTGTATTTGTATTTGTGTTTTGTTGTTGTGTCT
   imtsp : ------------------------------------------------------------

cipimtla : TTGTAGCTAAGTTGATATCCTGCTCATCTAGGCTGGCTGCATTTTTTTTGTGGCTGCCTG
   imtsp : ------------------------------------------------------------

cipimtla : ACAATGTAGCATTTGTGGTTTTCTTTCAATAAAGCATCTA.TTGTACCTCTGTTATCAGT
   imtsp : -G--------------------------------------t-------------------

cipimtla : GTATGATTTGCCTTTATTTTTAATAACTTAATTTTTTTTTCTTGTTTATATCCA
   imtsp : -----------------.....................................
```

FIG. 24
(continued)

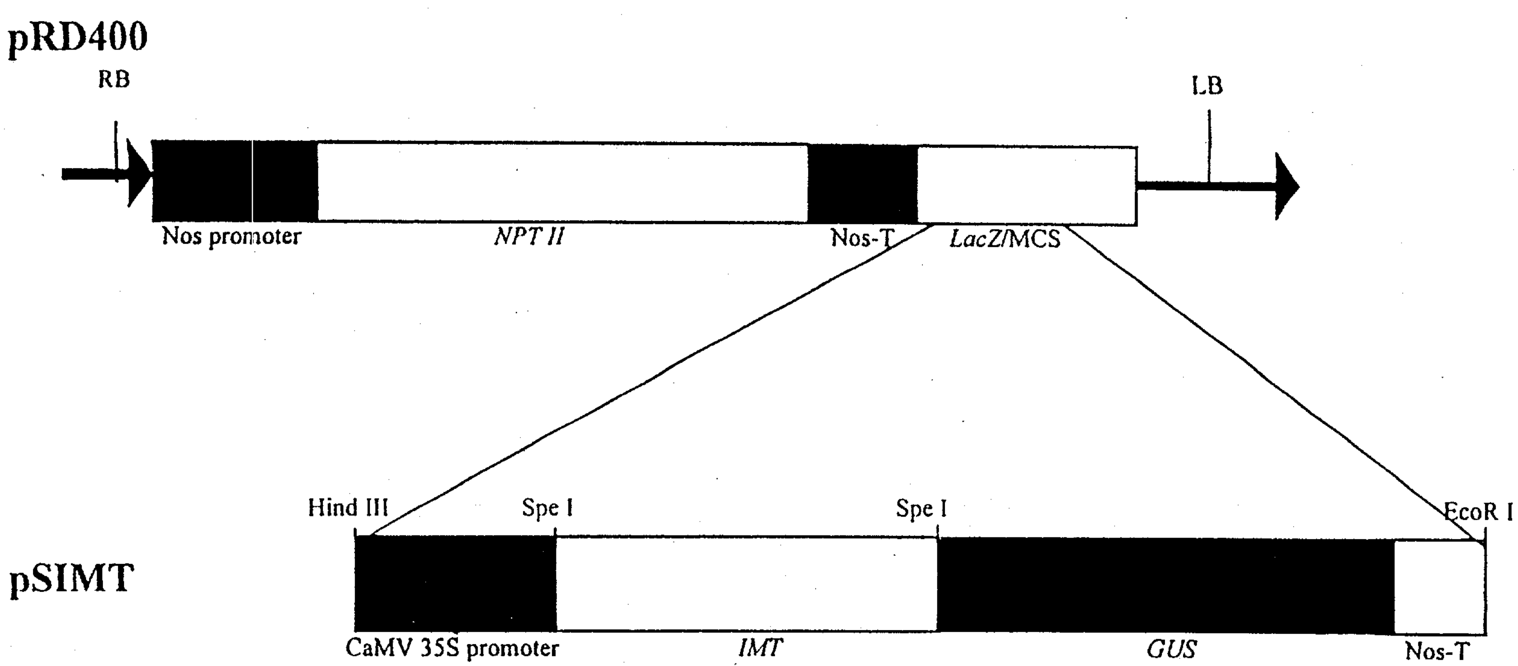

pRD400
RB
LB
Nos promoter
NPT II
Nos-T
LacZ/MCS
pSIMT
Hind III
Spe I
Spe I
EcoR I
CaMV 35S promoter
IMT
GUS
Nos-T

FIG. 25

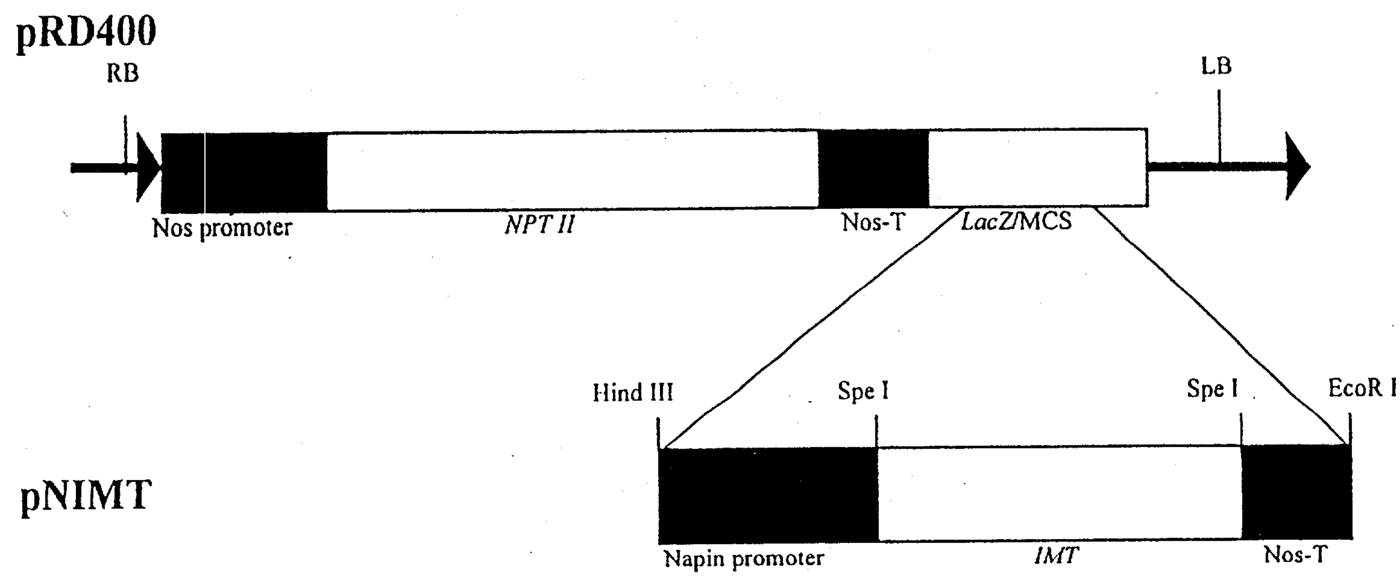

pRD400
RB
LB
Nos promoter
NPT II
Nos-T
LacZ/MCS
Hind III
Spe I
Spe I
EcoR I
pNIMT
Napin promoter
IMT
Nos-T

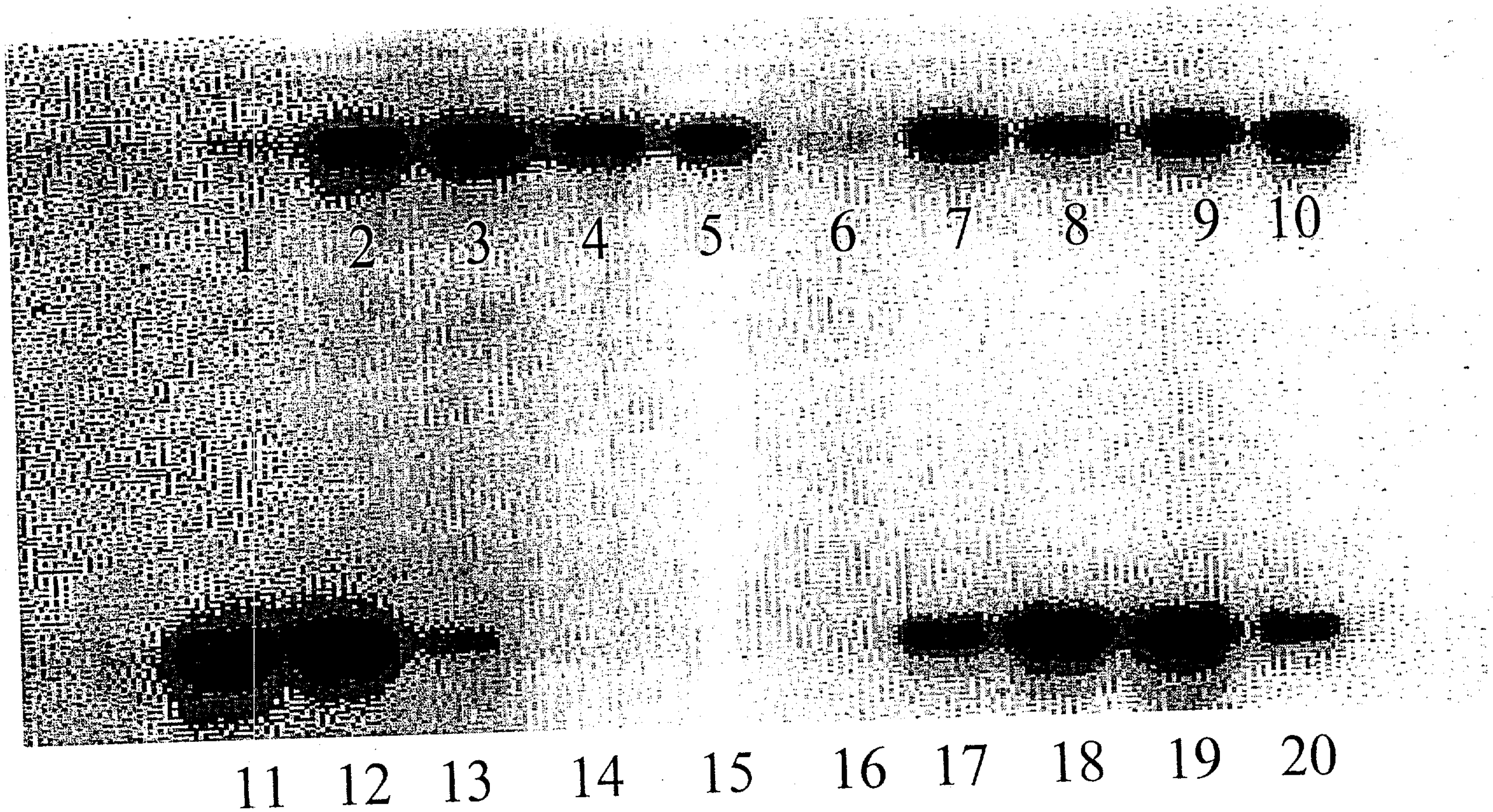
FIG. 26

PCR analysis of transgenic plants containing the *IMT* gene

M=molecular marker ;1= control plant ;2-22=transgenic plants

Figure 27

Northern blot analysis of 35S-IMT transgenic plants



1-20=individual transgenic plants

FIG. 28

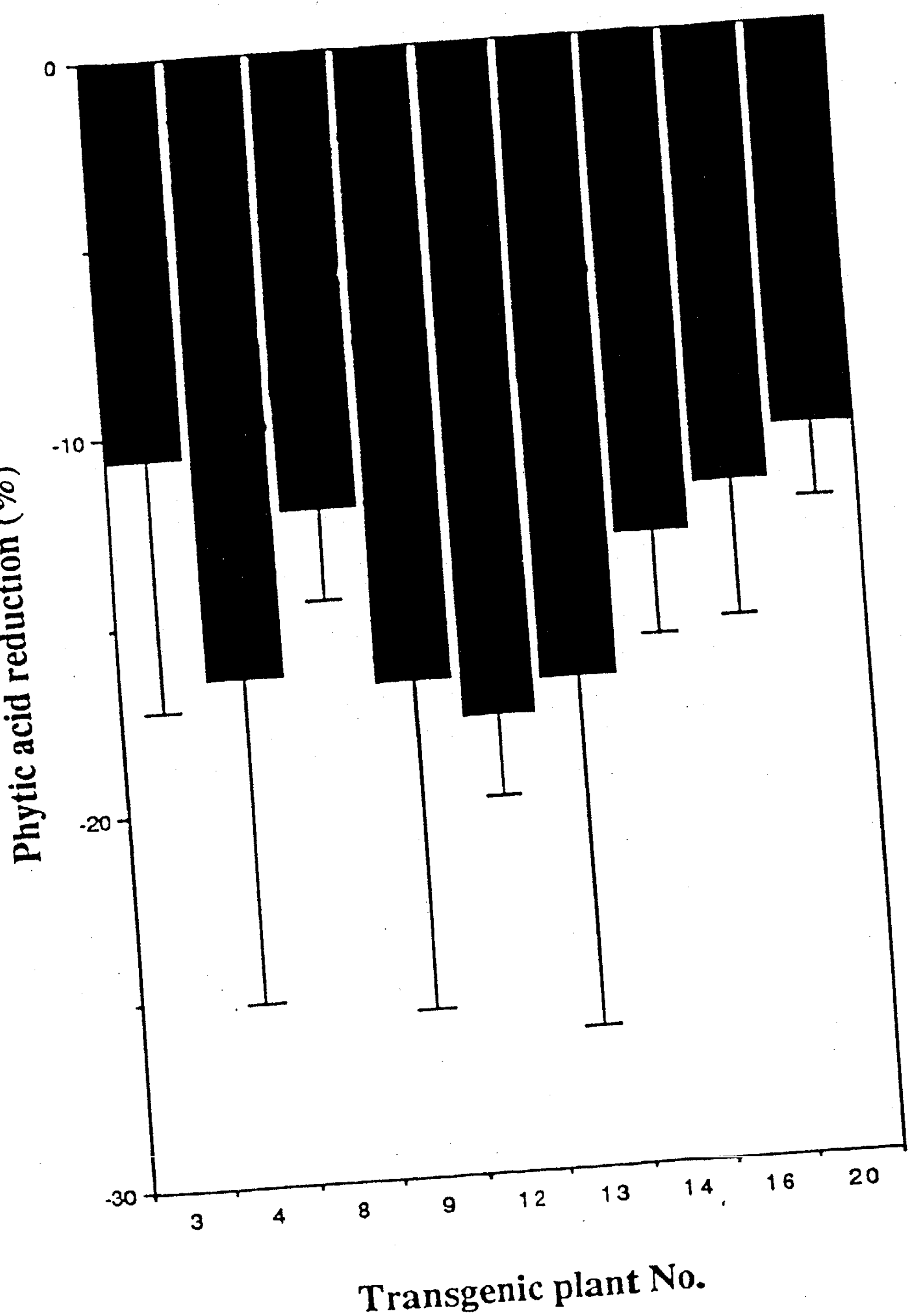

0
-10
-20
-30
Phytic acid reduction (%)
3
4
8
9
12
13
14
16
20
Transgenic plant No.

FIG. 29

Reduction of Phytic Acid in transgenic Plants

Percent Phytic Acid reduction in F1, F2 & F3 seeds from plants containing pSIMT

Transgenic Plant Number	Copy number of inserted gene	Percent Phytic Acid Reduction in F1 seeds	Percent Phytic Acid Reduction in F2 seeds	Percent Phytic Acid Reduction in F3 seeds
3	4	-10.5	-26	-36
6	3	-3.7	-24	-32
11	1	-8.6	-30	-34
17	3	-5.7	-29	-25

(a negative percent (-) means a reduction in phytic acid relative to non-transformed plants.)

FIG. 30

Reduction of Phytic Acid in transgenic Plants

Percent Phytic Acid reduction in F1 & F2 seeds from plants containing pNIMT

Transgenic Plant Number	Copy number of inserted gene	Percent Phytic Acid Reduction in F1 seeds	Percent Phytic Acid Reduction in F2 seeds
5	1	+24.31	-37
7	n.d.	-6.99	-44.81
12	2	-1.2	-39
15	1	-5.36	-24
17	2	-1.5	-38
19	3	-7.38	-43
21	1	-7.78	-37
N	1	+17.76	-31.54

(a negative percent (-) means a reduction in phytic acid relative to non-transformed plants.)

FIG. 31